

CLAIMS

We claim:

1. A functional TGF- β family fusion protein, comprising:
 - 5 a functionalizing peptide portion for detecting, quantifying or providing a specific additional function to the fusion protein; and a mature TGF- β family protein, or a variant or fragment thereof having at least 85% sequence identity with the mature TGF- β family protein and which retains TGF- β family protein activity.
- 10 2. A functional TGF- β family protein dimer formed by the association of two of the fusion proteins of claim 1.
- 15 3. The dimer of claim 2, wherein the dimer is a homodimer.
4. The dimer of claim 2, made by a process comprising:
 - 15 expressing a nucleic acid molecule in a eukaryotic cell to produce a monomer fusion protein, wherein the nucleic acid molecule comprises:
 - 20 a sequence encoding the functionalizing peptide portion;
 - a sequence encoding the mature TGF- β family protein; and
 - a sequence encoding a pro-region (latency associated peptide) of the TGF- β family protein, located to provide targeting and/or assembly and/or processing of the fusion protein encoded for by the nucleic acid.
- 25 5. The dimer of claim 4, wherein the process further comprises: associating two monomer fusion proteins to form the dimer.
6. The dimer of claim 4, wherein the sequence encoding the pro-region is located upstream to both the sequence encoding the functionalizing peptide portion and the sequence encoding the mature TGF- β family protein.

7. The dimer of claim 4, wherein the process further comprises:
cleaving the pro-region (latency associated peptide) from at least one fusion monomer.

5 8. The dimer of claim 4, wherein the process further comprises:
cleaving the pro-region (latency associated peptide) from both fusion monomers.

9. The fusion protein of claim 1, wherein the functionalizing peptide
10 portion is at the N-terminus of the mature TGF- β family protein.

10. The fusion protein of claim 9, wherein the mature TGF- β family protein is TGF- β 1.

15 *Jul B1* 11. The fusion protein of claim 10, where the protein comprises the
amino acid sequence as in SEQ ID NO: 11, SEQ ID NO: 15, the mature portion of
SEQ ID NO: 17, the mature portion of SEQ ID NO: 21, the mature portion of SEQ
ID NO: 33, the mature portion of SEQ ID NO: 35, the mature portion of SEQ ID
NO: 35, the mature portion of SEQ ID NO: 37, or conservative substitutions thereof.

20 12. The fusion protein of claim 1, wherein the functionalizing peptide portion is inserted within the mature functional TGF- β family protein.

13. The fusion protein of claim 12, wherein the functionalizing peptide
25 portion is inserted at a position of relatively low sequence conservation within the
TGF- β super family.

14. The fusion protein of claim 12, wherein the functionalizing peptide portion is inserted between a pair of adjacent residues between about residues 1 and
30 22 of the mature TGF- β family protein.

15. The fusion protein of claim 14, wherein the functionalizing peptide portion is inserted between residues 11 and 12 of the mature TGF- β family protein.

16. The fusion protein of claim 14, wherein the mature TGF- β family
5 protein is TGF- β 1.

17. The fusion protein of claim 16, where the protein comprises the amino acid sequence as in SEQ ID NO: 15, or conservative substitutions thereof.

10 18. The fusion protein of claim 1, further comprising a pro-region (latency associated peptide) of the TGF- β family protein located to provide targeting and/or assembly and/or processing of the fusion protein.

15 19. The fusion protein of claim 18, wherein the pro-region is located at the N-terminal region of the fusion protein.

20 20. The fusion protein of claim 1, wherein the mature TGF- β family protein is TGF- β 2, TGF- β 3, TGF- β 1, TGF- β 4 (chicken), TGF- β 5 (*Xenopus*), GDF-9 (mouse/human), BMP-16/nodal (mouse), Fugacin (*Xenopus*), BMP3, Sumitomo-
25 BIP/GDF-10 (mouse), ADMP (*Xenopus*), BMP-9, Dorsalin-1 (Chicken), BMP-10, BMP-13/GDF-6 (mouse), Radar (Zebrafish), GDF-1/CDMP-1 (mouse/human), BMP-12/GDF-7 (mouse), BMP-5, BMP-6, BMP-7/OP-1, BMP-8/OP-2, PC8/OP-3 (mouse), 60A (*Drosophila*), BMP-2, BMP-4, Decapentaplegic (*Drosophila*), Vg-1 (*Xenopus*), Univin (sea urchin), Vgr-2/GDF-3, GDF-1, Screw (*Drosophila*), BMP-11, GDF-8, Activin β C, Activin β D (*Xenopus*), Activin β E, BMP-14/GDF-12, Activin β A, Activin β B, GDF-14, Mullerian inhibiting substance, or α -inhibin.

21. The fusion protein of claim 1, wherein the mature TGF- β family protein is TGF- β 1, TGF- β 2, or TGF- β 3.

22. The fusion protein of claim 1, wherein the mature TGF- β family protein is TGF- β 1.

23. The fusion protein of claim 1, wherein the mature TGF- β family protein is TGF- β 2.

24. The fusion protein of claim 1, wherein the mature TGF- β family protein is TGF- β 3.

10 25. The fusion protein of claim 1, wherein the functionalizing peptide portion comprises a tag, a targeting moiety, or a biologically active protein domain.

26. The fusion protein of claim 25, wherein the targeting moiety comprises a domain of a cell surface binding protein.

15 27. The fusion protein of claim 25, wherein the biologically active protein domain comprises a toxin, an enzyme, or a fluorescent peptide.

28. The fusion protein of claim 25, wherein the tag is an epitope tag, a 20 purification tag, or an identification tag.

29. The fusion protein of claim 25, wherein the tag comprises a FLAG tag, a c-myc tag, a 6x His tag, a HA tag, a Tat tag, a T7 tag, a GFP peptide, or a GST peptide.

25 30. An isolated nucleic acid molecule encoding a fusion protein of claim 1, or a conservative substitution thereof.

Jul 88 31. The isolated nucleic acid molecule of claim 30, comprising a 30 sequence selected from the group consisting of:

(a) nucleic acid residues 835 to 1197 of SEQ ID NO: 8;

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- (b) SEQ ID NO: 10;
- (c) residues 835 to 1197 of SEQ ID NO: 12;
- (d) SEQ ID NO: 14;
- (e) residues 182-1559 of SEQ ID NO: 18;
- (f) residues 1182-1571 of SEQ ID NO: 22;
- (g) residues 907-1284 of SEQ ID NO: 24;
- (h) residues 914-1303 of SEQ ID NO: 26;
- (i) residues 895-1272 of SEQ ID NO: 28;
- (j) residues 895-1284 of SEQ ID NO: 30;
- (k) residues 845-1222 of SEQ ID NO: 32;
- (l) residues 849-1226 of SEQ ID NO: 34;
- (m) residues 845-1234 of SEQ ID NO: 36;
- (n) residues 845-1234 of SEQ ID NO: 38;

10

and

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- (o) conservative variants of any one of (a) through (n).

32. The isolated nucleic acid molecule of claim 30, further comprising a sequence encoding a TGF- β pro-region.

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33. The isolated nucleic acid molecule of claim 30, comprising a sequence selected from the group consisting of:

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- (a) SEQ ID NO: 8;
- (b) SEQ ID NO: 12;
- (c) SEQ ID NO: 18;
- (d) SEQ ID NO: 22;
- (e) SEQ ID NO: 24;
- (f) SEQ ID NO: 26;
- (g) SEQ ID NO: 28;
- (h) SEQ ID NO: 30;
- (i) SEQ ID NO: 32;
- (j) SEQ ID NO: 34;

(k) SEQ ID NO: 36; and
(l) SEQ ID NO: 38

34. A recombinant nucleic acid molecule comprising a promoter
5 sequence operably linked to the isolated nucleic acid molecule according to claim
30.

35. A transgenic cell comprising a recombinant nucleic acid molecule
according to claim 34.

10 36. The transgenic cell of claim 35, wherein the cell is a bacterial cell or
an eukaryotic cell.

15 37. The eukaryotic cell of claim 36, wherein the cell is a yeast cell or a
mammalian cell.

38. A transgenic organism comprising the transgenic cell of claim 35.

20 39. A method of adding a non-native functionality to a mature
biologically active TGF- β family protein, comprising
inserting a functionalizing peptide portion between a TGF- β pro-region and a
TGF- β mature protein, or at a relatively non-conserved site within the mature region
of a TGF- β family protein.

25 40. The method of claim 39, wherein the TGF- β family protein is TGF- β 2, TGF- β 3, TGF- β 1, TGF- β 4 (chicken), TGF- β 5 (*Xenopus*), GDF-9
(mouse/human), BMP-16/nodal (mouse), Fugacin (*Xenopus*), BMP3, Sumitomo-
BIP/GDF-10 (mouse), ADMP (*Xenopus*), BMP-9, Dorsalin-1 (Chicken), BMP-10,
BMP-13/GDF-6 (mouse), Radar (Zebrafish), GDF-1/CDMP-1 (mouse/human),
30 BMP-12/GDF-7 (mouse), BMP-5, BMP-6, BMP-7/OP-1, BMP-8/OP-2, PC8/OP-3
(mouse), 60A (*Drosophila*), BMP-2, BMP-4, Decapentaplegic (*Drosophila*), Vg-1

(*Xenopus*), Univin (sea urchin), Vgr-2/GDF-3, GDF-1, Screw (*Drosophila*), BMP-11, GDF-8, Activin β C, Activin β D (*Xenopus*), Activin β E, BMP-14/GDF-12, Activin β A, Activin β B, GDF-14, Mullerian inhibiting substance, or α -inhibin.

5 41. The method of claim 39, wherein the functionalizing peptide portion comprises a tag, a targeting moiety, or a biologically active protein domain.

10 42. A method of treating a disease that responds to administration of a TGF- β family protein, or assessing a pharmalogic property of the protein, comprising administering a therapeutically sufficient amount of the fusion protein of claim 1 to a subject.

15 43. The method of claim 42, wherein the functionalizing peptide portion is for detection or quantifying the fusion protein, and the method further comprises detecting or quantifying the fusion protein.

44. The method of claim 43, further comprising performing a pharmacokinetic or pharmacodynamic calculation.

20 45. The method of claim 44, wherein the calculation comprises a pharmacodynamic calculation of cellular site of protein action, dose-response relationship(s), structure-activity relationship(s), or quantitation of protein-receptor interaction(s).

25 46. The method of claim 44, wherein the calculation comprises a pharmacokinetic calculation of absorption, bioavailability, distribution, metabolism, or elimination/clearance of the protein.

30 47. The method of claim 44, wherein the method comprises detecting the fusion protein.

48. The method of claim 44, wherein the method comprises quantifying
the fusion protein. *B*

49. A purified functional TGF- β fusion protein, comprising an amino
5 acid sequence selected from the group consisting of:

(a) SEQ ID NO: 9;
(b) SEQ ID NO: 11;
(c) SEQ ID NO: 13;
(d) SEQ ID NO: 15;
(e) SEQ ID NO: 21;
(f) SEQ ID NO: 25;
(g) SEQ ID NO: 27;
(h) SEQ ID NO: 29;
(i) SEQ ID NO: 31;
15 (j) SEQ ID NO: 33;
(k) SEQ ID NO: 35;
(l) SEQ ID NO: 37;
(m) SEQ ID NO: 39;
(n) sequences having 85% sequence identity to any one of (a) through (m);
20 and
(o) conservative substitutions thereof.

50. An isolated nucleic acid molecule encoding the protein of claim 49.

25 51. A recombinant nucleic acid molecule comprising a promoter
sequence operably linked to the nucleic acid molecule of claim 50.

52. A transgenic cell comprising the recombinant nucleic acid molecule
according to claim 51.

53. The fusion protein of claim 9, wherein the mature TGF- β family protein is TGF- β 2.

54. The fusion protein of claim 53, where the protein comprises the 5 amino acid sequence as in the mature portion of SEQ ID NO: 25, the mature portion of SEQ ID NO: 27, or conservative substitutions thereof.

55. The fusion protein of claim 9, wherein the mature TGF- β family protein is TGF- β 3.

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56. The fusion protein of claim 55, where the protein comprises the amino acid sequence as in the mature portion of SEQ ID NO: 29, the mature portion of SEQ ID NO: 31, or conservative substitutions thereof.